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Interferon-inducible effector mechanisms in cell-autonomous immunity

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Abstract

Interferons (IFNs) induce the expression of hundreds of genes as part of an elaborate antimicrobial programme designed to combat infection in all nucleated cells — a process termed cell-autonomous immunity. As described in this Review, recent genomic and subgenomic analyses have begun to assign functional properties to novel IFN-inducible effector proteins that restrict bacteria, protozoa and viruses in different subcellular compartments and at different stages of the pathogen life cycle. Several newly described host defence factors also participate in canonical oxidative and autophagic pathways by spatially coordinating their activities to enhance microbial killing. Together, these IFN-induced effector networks help to confer vertebrate host resistance to a vast and complex microbial world.

Host effector mechanisms are essential for the survival of all multicellular organisms. This is exemplified by cell-autonomous immunity in plants, worms, flies and mammals. In *Arabidopsis* spp., for example, a definable set of resistance genes is mobilized during this programmed cell-intrinsic response to protect against diverse phytopathogens; this inherited response is sometimes referred to as the 'resistome'[1,2]. In higher species, however, the assembly of an antimicrobial arsenal or resistome takes on multiple forms, because the burden posed by infection in these organisms is considerable[3]. Indeed, as many as 1,400 phylogenetically distinct microorganisms can infect a single chordate host[4].

To cope with this increased microbial challenge, vertebrates have evolved additional levels of cell-autonomous control beyond the pre-existing repertoire of constitutive host defence factors. These additional factors include hundreds of gene products that are transcribed in response to signals originating from the interferon (IFN), tumour necrosis factor (TNF),

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interleukin-1 (IL-1) and Toll-like receptor (TLR) families[5,6]. Many of the induced proteins confer direct microbicidal immunity in all nucleated cells[7–9].

IFNs are among the most potent vertebrate-derived signals for mobilizing antimicrobial effector functions against intracellular pathogens[8,10,11]. Nearly 2,000 human and mouse IFN-stimulated genes (ISGs) have been identified to date, most of which remain uncharacterized (see the Interferome database)[12] (FIG. 1). The recent large-scale examination of newly described ISGs reveals a highly diverse but integrated host defence programme dedicated to protecting the interior of a vertebrate cell[13–16].

When viewed on a microscopic scale, the cell interior represents an immense ‘subterranean landscape’ to patrol and defend. A single human macrophage, for example, occupies ~5,000 μm^3 (REF. 17). Contrast this with a mature HIV-1 particle (~200 nm^3) or tubercle bacillus (~5–10 μm^3) and it quickly becomes apparent that most IFN-induced proteins will need to be dispatched to the site of pathogen replication to be effective[18,19]. Likewise, the ability of compartmentalized pathogens to remain largely sequestered in vesicles suggests that many IFN-induced effectors also need methods to detect these membrane-bound sanctuaries to eliminate the resident pathogens[18–20].

Several ISGs fulfil both criteria. Members of an emerging superfamily of GTPases with immune functions recognize specific host lipid molecules on the pathogen vacuole to mark it for disruption or delivery to lysosomes[21–23]. Other recently identified IFN-induced proteins detect ubiquitylated bacteria in the cytosol[24] or exposed glycans on host membranes that have been damaged by bacteria[25], and these markers stimulate the removal of the infecting organism through `autophagy`. In addition, new antiviral factors distinguish the cellular entry, replication and exit points of HIV-1 and influenza A viruses[13,15]. Less discriminating effector mechanisms are also deployed; for example, diatomic radical gases such as superoxide (O_2^-) and nitric oxide (NO) circumvent the need for recognition of the membranes surrounding sequestered bacteria and protozoa inside host cells[7,26]. Because such gases can diffuse large distances (several micrometres), they can also enter adjacent cells to confer *trans*-acting immunity, a property first noted for NO against herpes simplex virus, ectromelia virus and vaccinia virus[27]. Both of these strategies rely on an expanded family of oxidoreductases and peroxidases that is now known to be present in essentially all phyla[28].

It is the purpose of this Review to provide a broad conceptual framework for understanding IFN-induced cell-autonomous host defence and to highlight the growing list of effectors that combat internalized bacteria, protozoa and viruses at the level of the infected mammalian cell. It focuses principally on the downstream killing mechanisms, rather than on the well-known upstream microbial recognition and signalling events that elicit IFN production.

Cell-autonomous defence against bacteria

Bacteria infect host cells either through active invasion or via engulfment by professional phagocytes. Following their uptake, some bacterial species — such as *Mycobacterium tuberculosis* and *Salmonella enterica* serovars — inhabit membrane-bound compartments termed phagosomes, which they modify to limit their exposure to microbicidal factors[20]. By

contrast, *Chlamydia* spp. reside in reticulate structures called inclusion bodies, which intercept Golgi-derived exocytic traffic as a source of nutrition[29]. Other bacterial species, including *Listeria* and *Shigella* spp., escape their vacuoles to replicate in the cytosol. In each subcellular locale, IFN-induced effector mechanisms are mobilized to defend the interior of the host cell against bacterial infection. These mechanisms rely on oxidative, nitrosative and protonative chemistries, as well as nutriprive (nutrient-restrictive) and membranolytic activities.

IFN-induced oxidative and nitrosative defence

Cytotoxic gases are one of the most ancient and important forms of cell-autonomous defence. These gases — collectively termed `reactive oxygen species` (ROS) and `reactive nitrogen species` (RNS) — are generated by oxidoreductases to confer microbicidal activity and regulate intracellular signalling[7,26,28,30]. The targets of ROS and RNS include bacterial DNA (which is damaged via guanine base oxidation), lipids (which are damaged via peroxidation), and haem groups or iron–sulphur clusters within bacterial enzymes[7,26]. Much of the redox damage caused by these gases can be traced to derivatives of O_2^- and NO. For example, the sequential addition of single electrons to O_2^- yields hydrogen peroxide (H_2O_2) and then the hydroxyl radical ($^{\bullet}OH$), both of which are more powerful oxidants than their predecessor[26]. Likewise, the reaction of NO with O_2^-, other ROS or thiols yields intermediates with potent bactericidal properties: dinitrogen oxides (N_2O_3 and N_2O_4), compound peroxides ($ONOO^-$) and nitrosothiol adducts (RSNO)[7,26]. Within phagolysosomes, O_2^- undergoes spontaneous dismutation to H_2O_2, and stable nitrogenous end products such as nitrite (NO_2^-) are converted back at low pH to the volatile NO gas; both mechanisms aid bacterial killing[7,9].

Given the toxicity of these molecules, it is not surprising that the production of ROS and RNS is tightly controlled and often compartmentalized to limit self-injury. This has the added benefit of maximizing microbicidal activity when production is localized to phagosomes and `phagolysosomes` that contain bacteria[9]. In mammals, three classes of cytokine-inducible oxidoreductases control ROS and RNS production. `NADPH oxidases` (NOXs) directly catalyse the production of O_2^-, whereas dual oxidases (DUOXs) produce H_2O_2 (TABLE 1; Supplementary information S1 (figure)). In addition, nitric oxide synthases (NOSs) synthesize NO, and the immunologically inducible isoform NOS2 (also known as iNOS) synthesizes large amounts of NO under infectious conditions. All three classes of oxidoreductases may act simultaneously, sometimes even within the same host cell, depending on the physiological setting and the activating stimuli[7,28]. Non-enzymatic sources of ROS and RNS can also contribute to host defence. For example, O_2^- can originate from mitochondrial leakage and NO can be generated by the action of gastric acid on NO_2^- that is produced from dietary nitrates (NO_2^-) by the oral microbiota[7,28,31,32].

The NOX family of enzymes (NOX1 to NOX5) are the major ROS producers during infection[28]. NOX2 (also known as phagocyte oxidase) is responsible for the `respiratory burst` in neutrophils, monocytes, macrophages and eosinophils. Genetic evidence underscores its importance for host defence; indeed, congenital mutations in genes encoding NOX2 subunits give rise to a collective syndrome termed `chronic granulomatous`

disease. Affected individuals suffer from recurrent infections with catalase-positive organisms such as *Staphylococcus aureus*, *Serratia marcescens*, *Burkholderia cepacia*, non-typhoidal *Salmonella* spp. and *M. tuberculosis*[28,33] (TABLE 2).

NOX2 is a multisubunit enzyme comprising a transmembrane heterodimer — composed of gp91phox (also known as CYBB) and p22phox (also known as CYBA) — and three cytosolic subunits, namely p67phox (also known as NCF2), p47phox (also known as NCF1) and p40phox (also known as NCF4). The cytosolic subunits have SH3 domains that mediate intersubunit contacts and PX domains for binding membrane lipids once they translocate to the gp91phox–p22phox complexes at the plasma membrane or on plasma membrane-derived phagosomes[28] (TABLE 1) Supplementary information S1 (figure)). The assembly and activation of NOX2 holoenzymes also requires several GTPases. RAC1 and RAC2 facilitate this process under basal conditions[28], whereas the recently described GTPase guanylate-binding protein 7 (GBP7) operates after IFNγ stimulation[16]. IFNγ-induced GBP7 specifically recruits cytosolic p67phox-p47phox heterodimers to gp91phox–p22phox complexes on bacterial phagosomes containing *Listeria monocytogenes* or *Mycobacterium bovis* bacillus Calmette–Guérin (BCG)[16] (FIG. 2). The proximity of phagosomal NOX2 to intraluminal bacteria may heighten IFN-induced killing, as subsequent fusion with lysosomes favours dismutation of O_2^- to the more-damaging oxidant H_2O_2 (REF. 9). In addition to GBP7, the IFNγ-activated GTPase leucine-rich repeat kinase 2 (LRRK2) has recently been reported to promote NOX2 activity against *S.* Typhimurium[34]. How LRRK2 exerts its effects and whether it works in tandem with GBP7 on phagosomal membranes is currently unknown.

Other IFNγ-induced enzymes provide oxidative defence in non-phagocytic cells, such as epithelial cells lining the airways, oral cavity and gastrointestinal tract. The IFNγ-inducible enzymes NOX1 and DUOX2 generate O_2^- and H_2O_2, respectively, in these cells[28] (Supplementary information S1 (figure)). At the plasma membrane, H_2O_2 can form hypothiocyanite ($OSCN^-$), which acts as a potent chemorepellent against bacterial invasion and kills *Listeria* and *Salmonella* spp.[35–37]. Indeed, recent reports show that impaired clearance of *Salmonella* spp. follows the silencing of DUOX expression in zebrafish intestinal epithelium, indicating that such mechanisms operate during vertebrate immunity *in vivo*[38]. Thus, IFN-inducible NOXs and DUOXs restrict bacterial colonization not only of immune cells but also of stromal cells.

NOS2 is expressed in a variety of immune and non-immune cell types following stimulation by type I IFNs (that is, IFNα and IFNβ) and by IFNγ. Signals from other cytokines (notably, TNF, lymphotoxin-α and IL-1β) and from microbial products (such as lipopoly-saccharides and lipopeptides) also synergize with IFNs for NOS2 induction[7,39]. To date, most work has focused on NOS2 activities in mouse macrophages, as human mononuclear phagocytes produce lower NO levels[7]. Experiments using NOS2 inhibitors that are relatively selective for this NOS isoform have implicated a role for NO and its derivatives in the early cell-autonomous immune response to intracellular bacteria[7]. This role was further delineated in mice and macrophages deficient for NOS2 and/or gp91phox[39,40,41]. *M. tuberculosis* is sensitive to NO-mediated killing but relatively resistant to O_2^- and H_2O_2, in part owing to its expression of the H_2O_2-detoxifying enzyme KatG[42]. NO exhibits molar potencies

comparable to the current antibiotics used to treat tuberculosis, and the tuberculocidal activity of some new drugs (such as bicyclic nitroimidazoles) has been attributed to their release of NO[43]. By contrast, *L. monocytogenes* is sensitive to O_2^- and H_2O_2 but less vulnerable to NO, and *S. enterica* serovars are inhibited by both classes of chemicals[39,41]. Such differences reflect the metabolic pathways and microbial DNA repair processes targeted by ROS and RNS, as well as the detoxifying systems expressed by the bacteria[7] (see TABLE 2). They may also reflect compartmentalization; for example, *L. monocytogenes* becomes sensitive to NO when trapped inside phagosomes, owing to synergism with other bactericidal insults or the heightened RNS concentrations that accumulate in a confined volume[44]. Therefore, phagosomal escape of *L. monocytogenes* before NOS2 recruitment could provide a survival benefit for the pathogen. For this reason, vertebrates have evolved other IFN-induced mechanisms to deal with bacterial escapees, as discussed below.

Lysosomal killing: phagosome maturation and autophagy

Acidified lysosomes are inimical for the growth of most bacteria. Here, a low pH (~4.5–5.0) — which is generated via the action of proton-pumping vacuolar ATPases and maintained with the assistance of antiporters such as sodium/hydrogen exchanger 1 (NHE1) — enhances the bactericidal activity of both ROS and RNS[7,9]. In addition, an abundance of luminal proteases, lipases, glycosidases and antimicrobial peptides contributes to the sterilizing power of lysosomes[45,46]. This has resulted in some bacterial pathogens (such as *M. tuberculosis*) evolving strategies to avoid these degradative organelles, whereas other bacteria (such as *L. monocytogenes*) try to escape into the cytosol. Stimulation of the infected cell with IFNγ prevents both of these evasion strategies[18,22,44].

At least two newly described families of IFN-inducible GTPases — the 21–47 kDa immunity-related GTPases (IRGs) and the 65–73 kDa GBPs — traffic to vacuolar and cytosolic bacteria, where they assemble membrane complexes to facilitate bacterial transfer to lysosomes or disruption of the pathogen compartment[16,18,19,21–23]. IFN-inducible GTPases function together with three ubiquitin-binding receptors — sequestosome 1 (SQSTM1; also known as p62), NDP52 and optineurin — that detect ubiquitylated structures on bacteria, as well as with galectins that detect glycans that are exposed during bacterial escape into the cytosol. These receptors recruit the autophagic machinery that engulfs bacteria for lysosomal delivery[24,25,47–50]. The resultant (auto)lysosomes kill and degrade the entrapped cargo.

IRGs were first shown to target phagosomes and direct lysosomal membrane traffic in IFNγ-activated macrophages infected with *M. tuberculosis*[22]. It is now known that IRGs also exert membrane regulatory functions on other bacterial compartments, and their action has also been observed in human and mouse fibroblasts and epithelial cells[51–54]. IRGs promote cell-autonomous immunity to vacuolar bacteria as diverse as *M. tuberculosis, M. bovis, S.* Typhimurium, *Chlamydia trachomatis, Chlamydia psittaci, Legionella pneumophila* and Crohn's disease-associated adherent invasive *Escherichia coli* (AIEC)[22,23,51–61]. Individual IRGs confer pathogen-specific immunity *in vitro* and *in vivo*, indicating that they have non-redundant functions during host defence (TABLE 2). Such specificity probably arises from

the host-derived interacting partners and trafficking pathways used by a given IRG and the type of intracellular niche occupied by a given bacterial species[18,19,51].

Recent studies have contributed to a conceptual framework for how IRGs orchestrate immunity to different compartmentalized pathogens[21,23,53,54,59,60,62,63]. This model posits cooperative interactions between IRG subclasses, as well as with `SNARE proteins` and autophagic effectors that may disrupt the pathogen-containing compartment before lysosomal delivery (FIG. 2).

IRGs are divided into two groups — GKS-containing IRGs and GMS-containing IRGs — based on their canonical (lysine-containing) and non-canonical (methionine-containing) G1 motifs within the conserved amino-terminal catalytic GTPase domain[18,19,51] TABLE 1). IRGs in the GMS-containing subclass (IRGM1, IRGM2 and IRGM3 in mice; IRGM in humans) appear to be intrinsic regulators that control the activities of their respective effectors, which can also include other IRGs[21,23,53,54,59,60,62,63]. For example, IRGM3 in the endoplasmic reticulum (ER) helps to maintain membranolytic GKS-containing IRGs (such as IRGA6 and possibly IRGB10) in the ‘off’ state by acting as a non-canonical guanine nucleotide dissociation inhibitor (GDI)[62,63]. When released from IRGM3, IRGA6 and IRGB10 directly target *Chlamydia*-containing inclusion bodies or disrupt the trafficking of sphingomyelin-containing exocytic vesicles to these organelles[55,56]. Such disruption probably results in autophagic engulfment of the pathogen[53] and explains the susceptibility of *Irgm3*$^{-/-}$, *Irga6*$^{-/-}$ and *Irgb10*$^{-/-}$ fibroblasts to infection with *C. trachomatis* or *C. psittaci*[52,56,58].

IRGM1 and its smaller constitutive human orthologue, IRGM, engage their effectors when targeting *M. tuberculosis, M. bovis, S.* Typhimurium, AIEC or early *L. monocytogenes* phagosomes as part of the IFNγ-induced response to these bacteria[21–23,51,54,57,59,60]. The translocation of IRGM1 to mycobacterial phagosomes involves the recognition of specific host phosphoinositide lipids (namely, phosphatidylinositol-3,4,5-trisphosphate and, to a lesser extent, phosphatidylinositol-3,4-bisphosphate) on the nascent phagocytic cup[21] (FIG. 2). Once recruited, IRGM1 interacts with and may regulate the assembly activity or phosphorylation status of snapin, a SNARE adaptor protein that recruits dynein motor complexes to traffic phagosomes and endosomes along microtubules towards maturing autolysosomes[21,64]. Likewise, different human IRGM splice isoforms bind to the core autophagy proteins ATG5 and LC3B as well as the inner mitochondrial membrane lipid cardiolipin to induce mitochondrial fission and autophagy[59,65]; these functions of IRGM may underlie its protective response to mycobacterial, *Salmonella* spp. and AIEC infections[23,54,59,60,65,66]. Thus, a single GMS-containing IRG can act as a hub for coordinating membranolytic, fusogenic and fission events in an individual cell. This accounts in part for why deficiencies in GMS-containing IRGs cause such pronounced infectious phenotypes compared with those of GKS-containing IRGs[18,20,52,56–58] (TABLE 2). It may also explain why human *IRGM* polymorphisms share genetic linkages with susceptibility to tuberculosis and Crohn’s disease across so many geographically diverse populations[66–69].

In contrast to the IFN-inducible IRGs, GBPs target escaped bacteria in addition to those residing within vacuoles[16,70,71]. Nucleotide-dependent self-assembly of some but not all GBPs — in which G domain dimers pair with the carboxy-terminal helical domain (CTHD) to form GBP tetramers — helps to partition GBPs between the cytosol and endomembranes of the cell[16,72,73] (P. Kumar and J.D.M., unpublished observations) (TABLE 1). A C-terminal CaaX motif used for isoprenylation also contributes to this membrane attachment[16,72–74]. These structural features — along with an ability to oligomerize with other GBPs or even interact with GMS-containing IRGs — dictate which endomembranes individual GBPs occupy[16,72–76] and aid GBP targeting to both vacuolar and cytosolic bacteria (as the latter may retain remnants of damaged host membrane on the surface of their capsular coat following escape from the vacuole)[16] (C. J. Bradfield, P. Kumar and J.D.M., unpublished observations). This translocation of GBPs to bacteria promotes intracellular defence against *L. monocytogenes*, *S.* Typhimurium, *Chlamydia* spp. and *Mycobacterium* spp. in both macrophages and epithelial cells[16,70,71]. The lack of such cell-autonomous defence probably contributes to the susceptibility of *Gbp1*$^{-/-}$ and *Gbp5*$^{-/-}$ mice to bacteria[16] (TABLE 2).

The recent identification of interacting partners for GBP1 and GBP7 has begun to reveal the molecular mechanisms used by some of these GTPases to promote bacterial killing[16]. GBP1 interacts with the ubiquitin-binding protein SQSTM1, which delivers ubiquitylated protein cargo to autolysosomes, resulting in the generation of antimicrobial peptides that kill engulfed *M. bovis* and *L. monocytogenes*[16,77,78]. GBP7 recruits the autophagy protein ATG4B, which drives the extension of autophagic membranes around bacteria within damaged bacterial compartments and assembles NOX2 on these compartments[16] (FIG. 2). GBP5, by contrast, binds NLRP3 (NOD-, LRR- and pyrin domain-containing 3) to promote specific inflammasome responses during the infection of IFNγ-activated macrophages by *Listeria* or *Salmonella* spp., whereas in non-phagocytic cells heterotypic interactions between GBPs may help to target cytosolic escaped bacteria to autolysosomes (A. R. Shenoy, C. J. Bradfield and J.D.M., unpublished observations). Thus, GBPs act in concert — both temporally and physically — to confer their antibacterial effects. Moreover, they integrate oxidative, lysosomal and possibly inflammasome-related killing as part of their host defence activities.

In addition to being targeted by GBPs, cytosolic bacteria have recently been shown to encounter a second line of cell-autonomous defence orchestrated by SQSTM1, NDP52, optineurin and galectins in macrophages and epithelial cells[24,25,47–50]. The IFN-inducible proteins SQSTM1 and NDP52, along with basally expressed optineurin, recognize bacteria coated with ubiquitin, whereas IFN-regulated galectins detect the β-galactoside moiety of polysaccharide sugars (host glycans and microbial carbohydrates) that become exposed on damaged membranes when bacteria escape their phagosome to reach the cytosol[25,79–81]. SQSTM1, NDP52 and optineurin all possess a C-terminal domain for binding ubiquitin and an internal or N-terminal region that interacts with LC3 autophagy proteins for delivering bacterial cargo to autophagic vacuoles (FIG. 2; TABLE 1). Galactin 3 and galactin 8 contain carbohydrate-recognition domains, and galectin 8 binds to NDP52, which links the

recognition of sugar moieties on bacteria with the autophagic machinery further downstream[25] (FIG. 2; TABLE 1).

NDP52 also recruits the IκB kinase (IKK) family kinase TBK1 (TANK-binding kinase 1) to ubiquitin-coated bacteria via the adaptor proteins SINTBAD (also known as TBKBP1) and/or NAP1 (also known as AZI2)[24]. TBK1 in turn phosphorylates optineurin to increase its affinity for ubiquitin; in this way, NDP52 and optineurin may cooperate to protect against infection[24,47]. Furthermore, NDP52 and SQSTM1 use septin- and actin-dependent autophagic pathways to target cytosolic *Shigella* spp. and the small percentage of *S.* Typhimurium that escape their vacuole[48,49]. By contrast, autophagic delivery of non-motile *L. monocytogenes* mutants occurs via a different, as yet unspecified, route[49,50]. Because SQSTM1 activates a second antibacterial pathway involving diacylglycerol to induce the assembly of NOX2 complexes[82], parallels may be drawn with the GBPs, which induce both oxidative and autophagic pathways to confer cell-autonomous host defence.

Competing for intracellular cations

Facultative and obligate intracellular bacteria often have stringent metal cation requirements for growth inside mammalian host cells, which serve as a rich natural source of these chemical elements. As a result, IFN-induced mechanisms have evolved to restrict the intraphagosomal and cytosolic availability of Mn^{2+}, Fe^{2+} and Zn^{2+}, and to enhance the transport of Cu^{+} into the phagosome, as Cu^{+} helps to drive the formation of microbicidal ROS[83–85]. Indeed, the activation of macrophages by IFNγ lowers Mn^{2+}, Fe^{2+} and Zn^{2+} concentrations by ~2–6-fold and increases Cu^{+} levels by ~5-fold within mycobacterial phagosomes[86].

Part of the reduction in metal cation concentrations depends on a proton-dependent Mn^{2+} and Fe^{2+} efflux pump called natural resistance-associated macrophage protein 1 (NRAMP1; encoded by *Slc11a1*), which is upregulated by IFNγ[83,87]. NRAMP1 prevents ion sequestration specifically by phagosomal pathogens and competes with bacterial ion transporters for access to these nutritional metals[83] (FIG. 2). For example, the growth of *S.* Typhimurium mutants that lack *mntH* (which encodes an NRAMP1 homologue with a high affinity for Mn^{2+} and Fe^{2+}) or *sit ABCD* (which encodes a second Mn^{2+}-binding transport system) is attenuated in IFNγ-activated macrophages from mice that express the wild-type NRAMP1 efflux pump, but not in macrophages from congenic mice with a non-functional NRAMP1 efflux pump (derived from a defective *Nramp1*^{G169D} allele)[88]. Similarly, infection of macrophages by an *M. tuberculosis* strain lacking Mramp (another bacterial NRAMP1 homologue) leads to increased Mn^{2+} and Fe^{2+} concentrations within the phagosome, and this may reduce bacterial viability[89].

IFNγ stimulation also regulates other cation transport mechanisms, for example by inducing the relocation of the P-type ATPase Cu^{+} pump ATP7A to the phagosome, where it can transport Cu^{+} across the membrane to promote the generation of intraluminal hydroxyl radicals[85]. This again leads to intraphagosomal killing of bacteria. IFNγ stimulation concomitantly increases the expression of the Fe^{2+} exporter ferroportin 1 (also known as SLC40A1) at the plasma membrane, while decreasing transferrin receptor expression to

limit Fe^{2+} uptake; both mechanisms further restrict the growth of *S*. Typhimurium in macrophages[84].

In sum, synergistic IFN-inducible effector mechanisms are deployed in the cytosol and in diverse intracellular compartments to control bacterial infection. For example, IRGs, GBPs and recognition receptors help to direct vacuolar bacteria as well as 'marked' cytosolic bacteria to acidified autophagolysosomes. Low lysosomal pH, in turn, accelerates the dismutation of O_2^- to the more powerful oxidant H_2O_2, converts NO_2^- back to the toxic radical NO and drives hydroxyl radical formation with the aid of imported Cu^+. Together, these IFN-regulated proteins help to maximize oxidative, nitrosative, protonative and membranolytic damage to bacterial targets in the lysosome.

Cell-autonomous defence against protozoa

In vertebrates, many protozoa are obligate intracellular pathogens that depend on the host cell for specific amino acids and metal ions. The nutritional and safety needs of different parasites often dictate the type of compartment they inhabit (reviewed in REF. 90). For example, the apicomplexan parasite *Toxoplasma gondii* (which causes human toxoplasmosis) occupies a non-fusogenic vacuole that excludes most host-derived proteins, whereas the kinetoplastid parasites *Trypanosoma cruzi* (which is responsible for Chagas disease) and *Leishmania* spp. (which trigger cutaneous, mucocutaneous and visceral leishmaniasis) reside in the cytosol and in modified lysosomes, respectively[90]. These strategies operate effectively in resting cells by allowing the parasites access to nutrients while helping them to avoid contact with many host microbicidal proteins. However, once cells become stimulated with IFNs, new host defence pathways are transcriptionally induced to help limit parasite infection.

Parasiticidal activities

Previous studies have highlighted the role of NOS2-mediated killing in cell-autonomous defence against a variety of protozoa (reviewed in REF. 7). The parasiticidal effects of NO are most evident in IFNγ-activated macrophages infected with *Leishmania major* amastigotes or *T. cruzi* trypomastigotes and in human and mouse hepatocytes infected with *Plasmodium falciparum* and *Plasmodium yoelli* sporozoites, respectively[91–93](FIG. 3). Furthermore, *Nos2*$^{-/-}$ mice were highly susceptible to these pathogens[91–93] (TABLE 2). In the case of less virulent type II *T. gondii* tachyzoites, IFN-inducible NOS2 plays a more limited part, functioning at later time points[94] after the IFN-inducible GTPases have contained parasite growth during the early stages of infection[95]. For virulent type I *T. gondii* strains, however, NOS2 is essential, because these parasites have evolved mechanisms to escape IRG-mediated inhibition in IFNγ-activated macrophages[96]. Here, NO does not appear to eliminate virulent *T. gondii* but instead imposes static, non-lethal control[96]. How NO inhibits *Toxoplasma* parasites, along with malaria, *Leishmania* and *Trypanosoma* parasites, remains incompletely understood, but haem-containing compounds (such as haemozoin) and protozoal cysteine proteases appear to be likely targets for S-nitrosylation, which can inactivate these enzymes[7].

Targeting the parasitophorous vacuole

As in the case of bacteria, IFN-inducible IRGs and GBPs defend the interior of the host cell against protozoa. IRGM1, IRGM3 and IRGA6 promote IFNγ-induced control (but not TNF- or CD40-dependent control) of avirulent *T. gondii* in macrophages and astrocytes[96–101]. IRGM1 also contributes to macrophage trypanocidal activity[102] (TABLE 2). Inhibition of avirulent *T. gondii* appears to rely on several IRGs, with IRGM proteins providing a regulatory function by acting as GDIs that release GKS-containing IRGs to target the parasitophorous vacuole [FIG. 3). Recent studies invoke a hierarchical model in which IRGB6 and possibly IRGB10 act as forerunners to IRGA6 and then IRGD during their loading onto the parasitophorous vacuole some 90 minutes after parasite entry. The recruitment of these molecules is followed by vesiculation, membrane disruption and sometimes necroptosis[62,63]. What remains unknown are the structural and biochemical cues for targeting these molecules to the parasitophorous vacuole and whether membrane deformation is directly due to IRG activity or a result of some intermediary protein. These are topics of future investigation.

Other proteins assist the relocation of IRGs to the parasitophorous vacuole. For example, ATG5 facilitates the release and transit of IRGA6 from its bound state[103]. Heterotypic interactions between different GBPs have also recently been shown to underlie the vacuolar targeting of GBPs[76] (FIG. 3). Hence, multiple parasitophorous vacuole-damaging mechanisms are likely to ensue as the IRGs and GBPs converge on this organelle. Because virulent *T. gondii* strains (but not avirulent strains) exclude IRGs and GBPs from the parasitophorous vacuole[76,104,105], it is likely that these IFN-inducible GTPases exert a strong selective pressure via their membrane regulatory activities. Such pressure appears to be specific for different protozoa, as GBP1 is not recruited to *T. cruzi* compartments[76].

Restricting nutrient acquisition

Nutriprive mechanisms are particularly effective against parasites. NRAMP1 prevents ion assimilation by *Leishmania* spp. (*L. major* and *L. donovani*)[83] and indoleamine 2,3-dioxygenases (IDOs) hamper amino acid acquisition[106]. IDO1 and IDO2 are both IFN-inducible, haem-containing oxidoreductases that are responsible for the initial rate-limiting step of the kynurenine pathway, in which they degrade L-tryptophan to generate *N*-formylkynurenine [FIG. 3; TABLE 1). Removal of L-tryptophan restricts the growth of *Leishmania* spp. and *T. gondii* (as well as that of C. *psittaci*, *Francisella* spp., *Rickettsia* spp., herpes simplex virus 1 and hepatitis B virus) in IFNγ-activated macrophages, dendritic cells, fibroblasts, epithelial cells, astrocytes, endothelial cells and mesenchymal stem cells[107–111]. IDOs also inhibit *T. cruzi* via the downstream L-kynurenine catabolites 3-hydroxykynurenine and 3-hydroxyanthranilic acid, which are likely to be toxic for *T. cruzi* amastigotes and trypomastigotes[112]. Furthermore, *in vivo* blockade of IDOs using 1-methyl-tryptophan results in profound host susceptibility to *T. gondii*[113]. This host-protective role of IDOs against *T. gondii*, *Leishmania* spp. and *Chlamydia* spp. in humans is often superseded by the NOS2 pathway in other species (such as mice and rats), in which NOS2 may represent a more robust front-line defence mechanism[7,108,111].

Overall, the relative potencies of NOS2, IDOs, IRGs and GBPs against protozoa reflect not only the species-specific pathways available in vertebrates but also the co-evolutionary adaptations used by different parasites to survive within vertebrate host cells.

Cell-autonomous defence against viruses

Viruses were the first reported targets of IFN-mediated immunity, and they are the one taxonomic group that can infect all nucleated cells (reviewed in REF. 8). Less complex than eukaryotic protozoa and considerably smaller than most bacteria in terms of size and genome content, viruses nonetheless represent a major challenge to the host owing to their high mutation rates (up to 10^{-8} mutations per base per generation), their diverse cell tropisms and their ability to co-opt the replication machinery of the cell[8]. For these reasons, IFN-inducible proteins operate in multiple cell types and at all successive stages of the viral life cycle, including entry, replication, capsid assembly and release.

Blocking viral entry and uncoating

At least two IFN-inducible protein families have recently been shown to interfere with viral entry and uncoating: the IFN-inducible transmembrane (IFITM) proteins and the tripartite motif (TRIM) proteins.

IFITM1, IFITM2 and IFITM3 restrict the entry and endosomal fusion of influenza A virus and flaviviruses (such as West Nile virus and dengue virus) in both IFNγ- and IFNα-treated human cells[13]. Recent studies also extend the antiviral profile of these three IFITMs to include HIV-1, coronaviruses and the Marburg and Ebola filoviruses[114–116]. In the case of IFITM3, a C-terminal transmembrane region and S-palmitoylation contribute to its antiviral activity in membrane-bound compartments such as late endosomes and lysosomes[115–119] (TABLE 3). IFITM3 is thought to deny cytosolic access to influenza A virus by preventing viral genomes from leaving the endocytic pathway[119] (FIG. 4).

TRIMs also serve as viral restriction factors, particularly against retroviruses such as HIV-1. In vertebrates, many TRIMs are induced by IFNs (primarily by type I IFNs) in macrophages, myeloid dendritic cells, peripheral blood lymphocytes and fibroblasts[120]. TRIM-dependent antiviral activity relies on a shared N-terminal RING domain that functions as an E3 ligase and/or on a C-terminal SPRY domain that enables protein-protein interactions[120,121] TABLE 3). TRIM5a can restrict HIV-1 entry by binding to the retroviral capsid to accelerate its cytoplasmic uncoating and, as demonstrated more recently, by activating innate immune signalling through associations with the E2 ubiquitin-conjugating enzyme complex UBC13–UEV1A (also known as UBE2N–UBE2V1), which activates TGFβ-activated kinase 1 (TAK1) to induce immune genes[122,123]. Which of these two mechanisms predominates is as yet unresolved. In addition, TRIM22 combats hepatitis B virus and encephalomyocarditis virus by interfering with pre-genomic RNA synthesis and protease activity, whereas IFNβ-inducible TRIM79α restricts tick-borne encephalitis virus by mediating the lysosomal degradation of the viral RNA-dependent RNA polymerase NS5 (REFS 124–126). Furthermore, IFNα-inducible TRIM21 delivers incoming IgG-bound adenovirus to the proteasome through its E3 ubiquitin ligase activity[127]. Thus, the number of different effector mechanisms used by members of the TRIM family continues to grow.

The myxoma resistance proteins (MXs) are also antiviral effector molecules involved at an early stage in type I IFN- and IFNλ-induced host defence against orthomyxoviruses (such as influenza and Thogoto viruses), bunyaviruses, togaviruses and rhabdoviruses[128]. Human and mouse MX1, as well as mouse MX2, exhibit antiviral activity[128,129]. Mouse MX1 localizes to promyelocytic leukaemia (PML) nuclear bodies and restricts nuclear viruses, whereas both human MX1 and mouse MX2 are cytosolic proteins that target cytoplasmic viruses[128]. Human MX1 exhibits the broadest range of antiviral activity, targeting all the infectious genera of the *Bunyaviridae* family (that is, orthobunya-viruses, hantaviruses, phleboviruses and nairoviruses) as well as coxsackievirus and hepatitis B virus[128]. This fits with its expression in human endothelial cells, hepatocytes, plasmacytoid dendritic cells, peripheral blood mononuclear cells and other myeloid cells.

Current mechanistic models propose that GTPase-driven MX protein oligomers form ring-like structures to trap viral nucleocapsids and associated polymerases[128,130]. Such interactions may occur when MX proteins recognize incoming viral ribonuclear particle complexes that are destined for nuclear import or non-nuclear sites of replication[130]. Results from recent crystallography experiments suggest that disordered loops within an elongated MX1 helical ‘stalk’ may dock with negatively charged nucleocapsids to mediate entrapment[130].

Structural analogies with the MX proteins could also underpin the antiviral activity reported for dynamin-like GBPs against vesicular stomatitis virus (VSV), encephalomyocarditis virus, hepatitis C virus and influenza A virus[18,131]. Human GBP1, GBP3 and a novel splice isoform termed GBP3ΔC (which lacks part of the C-terminal helical domain) (TABLE 1) appear to be dependent on GTP binding but not hydrolysis for their effects, suggesting that oligomerization is important for the antiviral activity of GBPs. This evolutionary adaptation may allow GBPs to avoid viral antagonists such as the NS5B protein of hepatitis C virus, which can interfere with their catalytic activity[132].

Inhibiting viral replication

Once viruses uncoat, they establish cytoplasmic or nuclear sites of replication (which for *Retroviridae* includes chromosomal integration). The landmark discoveries of IFN-induced, RNA-activated protein kinase (PKR) and 2′–5′ oligoadenylate synthase 1 (OAS1), OAS2 and OAS3 (and OASL in humans) provided early insights regarding how viral RNA substrates are targeted (reviewed in REF. 8). PKR possesses RNA-binding motifs at its N-terminus that engage both double-stranded RNA (dsRNA) and single-stranded RNA (ssRNA) (TABLE 3); the viral uncapped RNAs that are recognized by PKR often have limited duplexed regions and 5′ triphosphate moieties, which enable the enzyme to distinguish them from host, capped RNA species[8]. Once activated, PKR phosphorylates eukaryotic translation initiation factor 2α (EIF2α) to block viral and host protein translation, a process that is thought to be under intense positive selection to avoid the emergence of viral mimics of the substrate EIF2α[133]. Likewise, the recognition of dsRNA by OAS enzymes results in the production of 2′-5′ oligoadenylates, which when polymerized activate the latent endoribonuclease RNase L to degrade viral RNA transcripts. Lastly, the

exonuclease ISG20 (IFN-stimulated gene 20 kDa protein) degrades RNA transcripts belonging to VSV, influenza virus and encephalomyocarditis virus[8].

Some IFN-dependent enzymes edit viral RNAs instead of degrading them. APOBEC3 (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide 3) and ADAR1 (adenosine deaminase, RNA-specific 1) are site-specific cytidine and adenosine deaminases, respectively. APOBEC3 converts cytidine to uridine in dsRNA, whereas ADAR1 catalyses the deamination of adenosine to inosine[8,134]. These incorporations lead to RNA destabilization and hypermutation after reverse transcription to cause lethal genome mutations in retroviruses such as HIV-1 (REF 135). Different APOBEC isoforms (APOBEC3F and APOBEC3G) exhibit distinct mechanisms involving the processing of long terminal repeats, and they may also interact with RNA and/or the Gag protein from HIV-1 to prevent the packaging of these molecules into viral particles[8,135] (FIG. 4).

Another IFN-inducible retroviral restriction factor termed SAM-domain- and HD-domain-containing protein 1 (SAMHD1) was found more recently in macrophages and dendritic cells[136–138], providing some explanation as to why HIV-1 inefficiently transduces mononuclear phagocytes. SAMHD1 contains a nucleotide-phosphohydrolase domain that hydrolyses deoxynucleotides from the cellular pool (TABLE 3), and depleting this nucleotide supply is currently posited to limit HIV-1 reverse transcriptase activity[138] (FIG. 4).

Non-nucleotide targets are also subject to IFN-mediated inhibition. The ubiquitin-like modifier ISG15 restricts influenza viruses, herpesviruses, Sindbis virus, HIV-1, human papillomavirus (HPV) and Ebola virus in cells activated by type I IFNs[139–141], and many of these viruses cause lethal infection in *Isg*15$^{-/-}$ mice[139]. ISG15 acts by conjugating target viral (and cellular) proteins in a process termed `ISGylation`[140]. ISGylation substrates include many newly synthesized viral proteins, such as the influenza A virus protein NS1 and the HPV capsid proteins L1 and L2, which are needed for replication and host evasion, and the HIV-1 protein Gag and the Ebola virus protein VP40, which are involved in viral budding[140,141]. ISGylation can interfere with modification of these viral proteins by ubiquitin, which would otherwise help to activate their functions[141].

Nitrosylation is another post-translational modification that inhibits viruses. NO released by IFN-induced NOS2 blocks DNA viruses — including poxviruses (such as ectromelia virus and vaccinia virus), herpesviruses (such as HSV-1 and Epstein-Barr virus) and rhabdoviruses (such as VSV) — as well some RNA viruses (such as coxsackie B3 virus)[7,27,142–144]. Where examined, the loss of antiviral effector function in *Nos2*$^{-/-}$ mice coincided with heightened susceptibility to viral infection (TABLE 2). The processes targeted by NO include early and late viral protein synthesis, as well as *S*-nitrosylation of structural proteins (in the case of VSV) or cysteine proteases (in the case of coxsackie B3 virus). They also extend to DNA replication (in the case of vaccinia virus) and to RNA or DNA synthesis via the inhibition of an immediate-early gene encoding the transactivator Zta (in the case of Epstein-Barr virus)[27,142–144]. Thus, replicative viral DNA and RNA, as well as viral proteins, serve as direct targets for IFN-mediated modification and inactivation.

Preventing viral assembly, budding and release

Following replication, viral DNA, RNA and structural proteins are packaged into nascent virions for budding and release. At least two recently described IFN-induced proteins — tetherin and viperin — affect late-stage export.

Tetherin (also known as CD317 and BST2) is a viral restriction factor that prevents the release of HIV-1 particles from infected macrophages, where it also serves as a target for the HIV-1 protein Vpu[145,146]. In addition, it prevents the release of filovirus, arenavirus and herpesvirus particles in response to type I IFN or IFNγ stimulation[147] in macrophages and plasmacytoid dendritic cells.

The mature tetherin protein is a type II transmembrane disulphide-linked dimer. Its C-terminal ecto-domain is modified by a glycophosphatidylinositol (GPI) linkage, and its N-terminal cytoplasmic domain contains YxY motifs for binding the clathrin adaptor proteins AP1 and AP2 during the endocytic internalization of tethered virus for lysosomal delivery[147] (TABLE 3). This topology may enable the association of tetherin with lipid rafts and virion lipids so that it can be incorporated into HIV-1 particles. The secondary rather than primary structure of tetherin is thought to dictate its antiviral activity[148], with the N-terminal and coiled-coil regions within the tetherin ectodomain minimally required for viral retention[149] (FIG. 4; TABLE 3).

Viperin (also known as RSAD2) was originally shown to be induced by type I and II IFN signalling in human cytomegalovirus-infected skin cells and in mice infected with lymphocytic choriomeningitis virus[150]. Viperin contains an S-adenosyl methionine (SAM) domain and an N-terminal amphipathic helix that contributes to its antiviral activity by helping viperin to associate with ER membranes or lipid droplets[151,152] (FIG. 4; TABLE 3), where it interferes with the assembly and egress of influenza virus and hepatitis C virus particles. This may occur through the disruption of ER-derived lipid rafts that transport viral envelope proteins to the plasma membrane, possibly via the inhibition of farnesyl pyrophosphate synthase, which is involved in cholesterol and isoprenoid synthesis[151,152]. Recent work also demonstrates that viperin inhibits dengue virus, HIV-1 and West Nile virus, although whether it uses similar mechanisms remains untested[150,153].

Numerous IFN-inducible restriction factors therefore target each stage of the viral life cycle in a variety of cell types, ensuring broad protective coverage to combat this diverse group of pathogens.

Conclusions and future directions

An avalanche of information has emerged over the last 15 years on the sensory apparatus and signalling cascades that mobilize innate immunity in response to infection[6]. By contrast, little is known about the cell-autonomous effector mechanisms that confer sterilizing immunity. How do we actually kill intracellular pathogens, or at least restrict their growth? Remarkably, such mechanisms seem to operate across most vertebrate cells, an inheritance foretold by the defence repertoires of plants and lower organisms[1,2], but with the added

features of expansive diversification and induction by IFNs in larger, long-lived chordates[3,8].

Recent applications of systems biology have begun to unearth new IFN-induced antiviral factors (such as IFITMs)[13], and genome-wide *in silico* identification coupled with traditional loss-of-function approaches has revealed proteins with novel antibacterial activities (such as GBPs)[16]. This list will continue to grow as we probe the interface between vertebrate hosts and microbial pathogens using large-scale unbiased methods[154,155], in some cases with the assistance of government centres dedicated to the systematic study of infection (see REF. 156).

As next-generation informatics takes hold, we are likely to find new IFN-inducible proteins with unique and perhaps unusual functions in host defence. For example, such proteins could protect the nucleus from retroviral insertion or bacterial factors[157]; defend gap junctions from bacterial cell-to-cell spread[158,159]; alter microbial or host cell metabolism[160]; participate in pathogen-selective forms of autophagy[161]; or use different forms of nucleotide-directed defence (such as `microRNAs` or interference with small non-coding microbial RNAs) instead of protein activity[162]. Such candidates would expand the reach of IFNs beyond toxic gases, lytic peptides, ion transporters, DNases and RNases as the main cell-intrinsic means by which to bring infection under control. They may also reinforce the idea that synergy between IFN-induced genes is more than the sum of their individual parts, one of the founding doctrines of systems biology[154–156].

Other outstanding questions include the identity of the membrane signals, signatures and structures that allow the recruitment of effectors to intact or damaged pathogen compartments for their eventual removal, a topic in which the IFN-inducible IRGs and GBPs will play a leading part. In fact, it was previously proposed that these and related proteins could provide a physical bridge between the detection and disposal of this particular class of organisms[18,163]. Now is the time to test such predictions by modern methods. To do so should help to build a more complete picture of intracellular defence at the single-cell level. It will also better define what constitutes the IFN-induced resistome.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Autophagy	A specialized process involving the degradative delivery of a portion of the cytoplasm or of damaged organelles to the lysosome. Internalized pathogens can also be eliminated by this pathway
Reactive oxygen species	(ROS). Aerobic organisms derive their energy from the reduction of oxygen. The metabolism of oxygen, and in particular its reduction through the mitochondrial electron-transport chain, generates by-products such as superoxide (O_2^-) and downstream intermediates such as hydrogen peroxide (H_2O_2 and hydroxyl radicals ($^{\bullet}OH$). These three species are referred to as ROS. ROS can damage important intracellular targets such as DNA, lipids or proteins
Reactive nitrogen species	(RNS). Nitric oxide (NO) chemistry is complex because of the extreme reactivity of NO, which can result in the formation of different reactive nitrogen intermediates (RNP depending on the amount of NO that is produced by cells. At low Concentrations, NO reacts directly with metals and other radicals. At higher concentrations, indirect effects prevail, and these include several oxidation or nitrosylation reactions with oxygen that result in the production of various congeners. NO and related RNI are effective antimicrobial agents and signal-transducing molecules
Phagolysosomes	Intracellular vesicles that result from the fusion of phagosomes, which enclose extracellular material that has been ingested, with lysosomes, which contain lytic enzymes and antimicrobial peptides
NADPH oxidases	Enzyme systems that consist of multiple cytosolic and membrane-bound subunits. The complex is assembled in activated phagocytic cells on the plasma and phagosomal membranes. NADPH oxidase uses electrons from NADPH to reduce molecular oxygen to form superoxide anions. Superoxide anions are enzymatically converted to hydrogen peroxide, which in neutrophils can undergo further conversion by myeloperoxidase to hypochlonc acid, a highly toxic and microbicidal agent
Respiratory burst	The process by which molecular oxygen is reduced by the NADPH oxidase system to produce reactive oxygen species
Chronic granulomatous disease	An inherited disorder caused by defective oxidase activity in the respiratory burst of phagocytes. It results from mutations in any of five genes that are necessary to generate the superoxide radicals required for normal phagocyte function. Affected patients suffer from increased susceptibility to recurrent infections
Galectins	Lectins that bind a wide variety of glycoproteins and glycolipids containing β-galactoside. They have extracellular and intracellular

	functions, including the regulation of apoptosis, RAS signalling, cell adhesion and angiogenesis
SNARE proteins	(Soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor proteins). A class of proteins that is required for membrane fusion events that occur in the course of vesicle trafficking and secretion
ISGylation	The attachment of the ubiquitin-like modifier ISG15 to either pathogen or host protein targets to regulate their function rather than stimulate degradation
Micro RNAs	Single-stranded RNA molecules of approximately 21–23 nucleotides in length that are thought to regulate the expression of other genes

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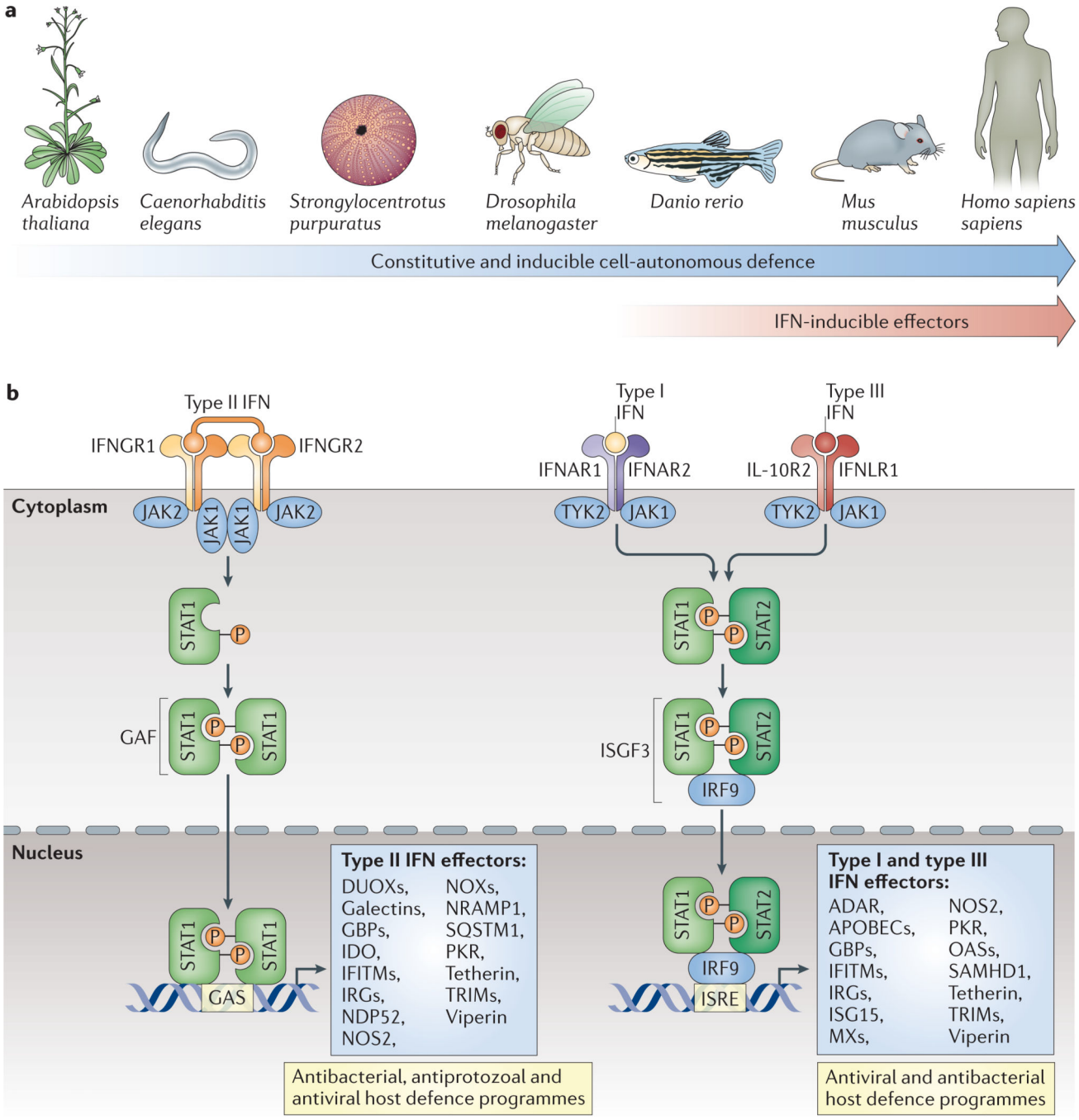


Figure 1. Evolution of IFN-induced cell-autonomous host defence

a The evolution of cell-autonomous immunity and the emergence of interferon (IFN)-induced effector mechanisms around the protochordate–vertebrate split (~530 million years ago). **b** Cell-autonomous host defence proteins are canonically induced by I FNs via three receptor complexes with high affinities for their ligands(K_α < 10 nM^{-1})[8]. The first receptor complex is a tetramer—composed of two chains of IFNγ receptor 1 (IFNGR1) and two chains of IFNGR2—that engages type II IFN (that is, IFNγ)dimers. The second is a heterodimer of IFNα/β receptor 1 (IFNAR1) and IFNAR2 that binds to the type I IFNs: a

family consisting of 13 different IFNα subtypes and one IFNβ subtype in humans. In the third receptor complex, interleukin-10 receptor 2 (IL-10R2) associates with IFNλ receptor 1 (IFNLR1; also known as IL-28Rα) to bind to three different type III IFN (that is, IFNλ) ligands (see REF. 8). Following receptor–ligand engagement, signals are transduced through signal transducer and activator of transcription 1 (STAT1) homodimers in response to IFNγ or through STAT1–STAT2 heterodimers in response to type I IFNs or IFNλ. Following their recruitment to the receptor complexes, these STAT molecules are phosphorylated by receptor-bound tyrosine kinases (namely, Janus kinases (JAKs) and tyrosine kinase 2 (TYK2)). Phosphorylated STAT1 homodimers (also known as GAF) translocate to the nucleus to bind to IFNγ-activated site (GAS) promoter elements to promote the IFN-induced expression of antimicrobial effector genes, some of which also require transactivation by IFN-regulatory factor 1 (IRF1) and IRF8. In the case of type I and III IFN signalling, phosphorylated STAT1-STAT2 dimers form a complex with IRF9 to yield IFN-stimulated gene factor 3 (ISGF3); this complex also translocates to the nucleus, where it binds to IFN-stimulated response elements (ISREs) in the promoters of different or overlapping IFN-stimulated effector genes.

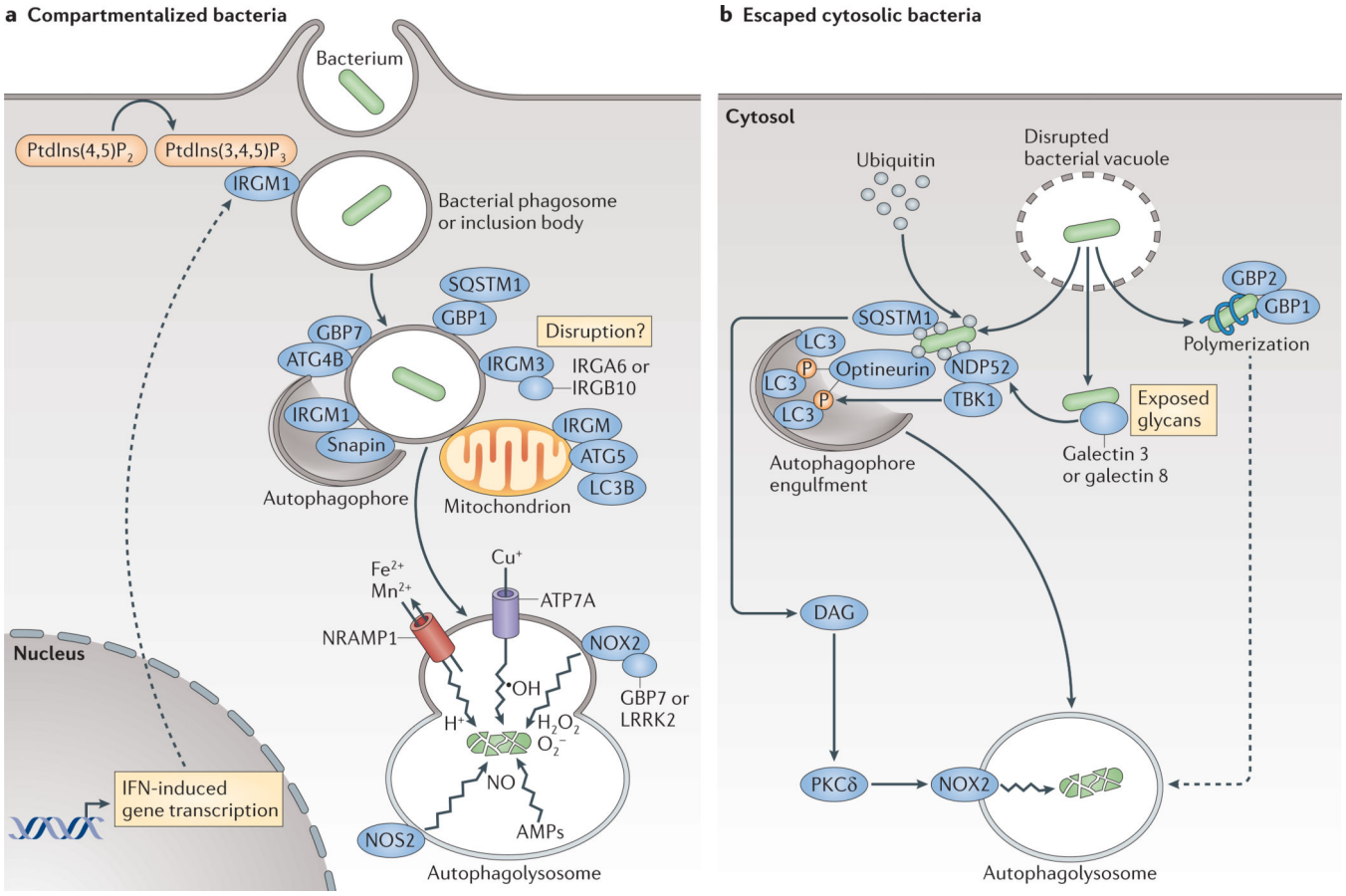


Figure 2. Cell-autonomous mechanisms used by IFN-induced proteins against intracellular bacteria

Interferon (IFN)-inducible proteins are required for host resistance to intracellular bacteria, **a** Specific immunity-related GTPases (IRGs).guanylate-binding proteins (GBPs) and other GTPases translocate to compartmentalized bacteria in phagosomes or inclusion bodies. Here, different membrane regulatory complexes — IRGM1-snapin, GBP1-sequestosome 1 (SQSTM1) and GBP7–ATG4B — are assembled. These complexes initiate autophagic capture and SNARE-mediated fusion of the bacterial compartments with lysosomes[16,21–23]. In addition, IRGM3-IRGA6 (or IRGB10) mediate vacuole disruption[19,52,53], and GBP7 (and possibly leucine-rich repeat kinase 2 (LRRK2)) help to assemble NADPH oxidase 2 (NOX2) on bacterial phagosomes, which mediates bacterial killing. Using this pathway, these GTPases can also deliver antimicrobial peptides (AMPs) to the autophagolysosome and, in the case of human IRGM, may instigate mitochondrial fission before autophagy[59]. Other IFN-inducible components, such as natural resistance-associated macrophage protein 1 (N RAMP1), help to exclude Mn^{2+} and Fe^{2+} from the bacterial phagosome, while importing protons (H^+) into this compartment. Nitric oxide synthase 2 (NOS2), which synthesizes NO, works in concert with NOX2, which produces reactive oxygen species such as superoxide (O_2^-)and hydrogen peroxide (H_2O_2), to produce compound intermediates like peroxynitrite (not shown) that are highly bactericidal. **b** An emerging signature for the recognition of some escaped bacteria in the cytosol is ubiquitylation (eithersingle or multiple modifications with monoubiquitin and/or polyubiquitin chains) (see REF. 47). SQSTM1,

NDP52 and optineurin bind to ubiquitylated bacteria to initiate innate immune signalling and to recruit the autophagic machinery via LC3 family members. In addition, GBP1 and GBP2 polymerize around cytosolic bacteria in a ubiquitin-independent process that may recruit specific antimicrobial partners, while galectin 3 and galectin 8 bind to exposed glycans on the bacteria and, in the case of galectin 8, recruit NDP52 and downstream autophagic effectors[25]. SQSTM1 also activates a second antibacterial pathway involving diacylglycerol(DAG)and protein kinase Cδ(PKCδ)to induce NOX2 complex assembly. Dashed lines indicate possible routes and consequences. Ptdlns(4,B)P_2, phosphatidylinositol-4,B-bisphosphate; Ptdlns(3,4,B)P_3,phosphatidylinositol-3,4,B-trisphosphate;TBK1,TANK-binding kinase 1.

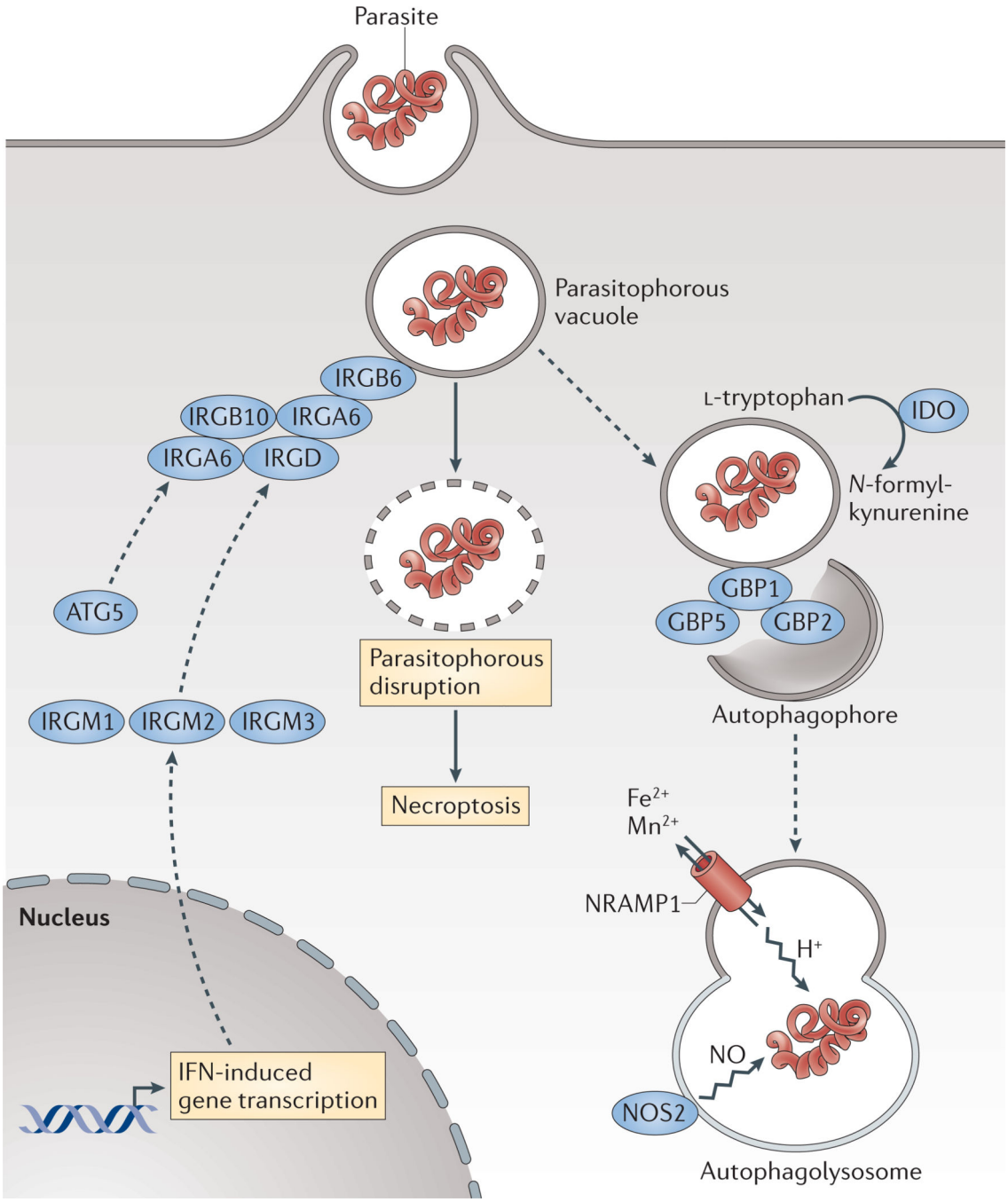


Figure 3. Cell-autonomous mechanisms used by IFN-induced proteins against intracellular protozoa

Different intracellular strategies are used by interferon (IFN)-inducible proteins against protozoa. Nitric oxide synthase 2 (NOS2) exerts potent parasiticidal activity, while GKS-containing immunity-related GTPases(IRGs) appear to be directly involved in parasite vacuole disruption once they reach the parisitophorous compartment. This proceeds via autophagy-independent trafficking after release from IRGM1–IRGM3 or ATG5 and is mediated by cooperative IRG loading[61,63,103]. Guanylate-binding proteins (GBPs) — specifically GBP1-GBP2 and GBP1-GBPB complexes — also traffic to the parasitophorous

vacuole, with uncharacterized effects on parasite control[76]. Natural resistance-associated macrophage protein 1 (NRAMP1) is important for restricting the uptake of Mn^{2+} and Fe^{2+} by this compartment, whereas indoleamine 2,3-dioxygenase 1 (IDO1) and/or IDO2 limit amino acid acquisition via the depletion of L-tryptophan. Dashed lines indicate possible routes or consequences.

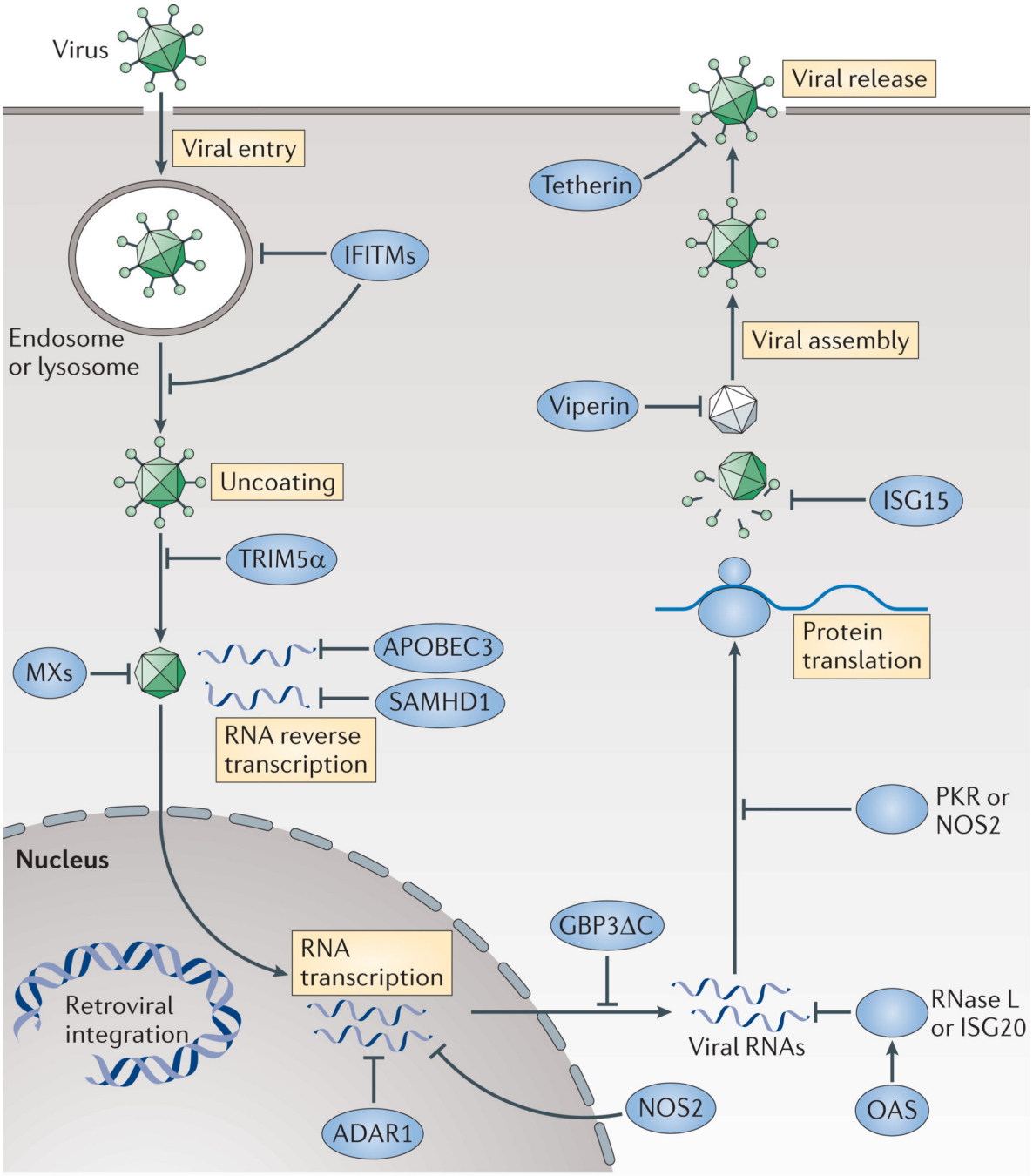


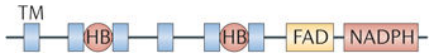
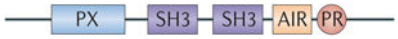
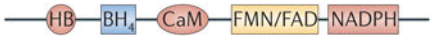
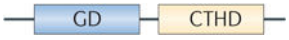
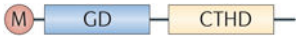
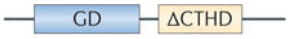
Figure 4. Cell-autonomous mechanisms used by IFN-induced proteins against viruses

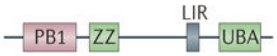
Multiple strategies are used by interferon (IFN)-inducible proteins to combat viruses. IFN-inducible effectors function at nearly every stage of the pathogen life cycle. For example, interferon-inducible transmembrane proteins (IFITMs) and tripartite motif proteins (TRIMs) act during viral entry and uncoating.and myxoma resistance proteins (MXs) block nucleocapsid transport. Inhibition of RNA reverse transcription, protein translation and stability is mediated by APOBEC3 (apolipoprotein B mRNA-editing enzyme, catalytic polypeptide 3), SAMHD1 (SAM-domain- and HD-domain-containing protein 1), ADAR1

(adenosine deaminase, RNA-specific 1), NOS2 (nitric oxide synthase 2), OASs (2′-5′ oligoadenylate synthases), RNase L, ISG20 (IFN-stimulated gene 20 kDa protein), PKR (IFN-induced, RNA-activated protein kinase) and ISG15. Finally, viperin and tetherin help to prevent viral assembly and release, respectively. Some of the effectors (such as MX proteins) appear to operate in both the nucleus and the cytosol (not shown).

Table 1

IFN-induced effector molecules that combat intracellular bacteria and parasites

IFN-induced effector	Member or subunit	Domain structure
NOX family	gp91phox	
	p22phox	
	p67phox	
	p47phox	
	p40phox	
	NOXA1	
	NOXO1	
DUOX family	DUOX1	
	DUOX2	
	DUOXA1 or DUOXA2	
NOS family	NOS2	
IRG family	Human IRGM (a to e isoforms)*	
	Mouse IRGM1 to IRGM3	
	IRGA,IRGB,IRGCorlRGD groups	
GBP family	Human GBP1 to GBP6 and mouse GBP1 to GBP11	
	Human GBP3ΔC	
NRAMP family	NRAMP1	
IDO family	ID01 or lD02	
Galectin family‡	Galectin 3	
	Galectin 8 or galectin9	

IFN-induced effector	Member or subunit	Domain structure
Ubiquitin-binding receptors‡	SQSTM1	
	NDP52	

AIR, autoinhibitory region; BH, tetrahydrobiopterin-binding domain; CaM, calmodulin-binding domain; CC, coiled–coil; CTHD, C-terminal helical domain; CRD, carbohydrate–recognition domain; DUOX, dual oxidase; EF, EF hand domain; FAD, flavin adenine dinucleotide binding site; FMN/FAD, flavin mononucleotide or flavin adenine dinucleotide binding site; GBP, guanylate–binding protein; GD, GTPase domain; HB, haem–binding site; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; IRG, immunity-related GTPase; LIR, LC3-interacting region; LIZ, LC3-interacting zipper; M, myristoylation site; NADPH, nicotinamide adenine dinucleotide phosphate binding site; NLD, non-lectin domain; NOS, nitric oxide synthase; NOX, NADPH oxidase; NRAMP, natural resistance-associated macrophage protein; OR, oxidoreductase domain; P, isoprenylation site; PB1, phox and Bem1 domain, PC, phox and Cdc domain; PerD, peroxidase domain; PR, proline-rich domain; PX, phox domain for phospholipid binding; RR, arginine-rich domain; SH3, SRC homology 3 domain; SQSTM1, sequestosome 1; TM, transmembrane domain; UBA, ubiquitin-associated domain; ZZ, zinc fingers.

* Human IRGM is constitutively expressed but participates in IFN-induced cell-autonomous immunity.

‡ Denotes indirect effectors that function via autophagy (only IFN-inducible receptors are shown).

Table 2

Genetic deficiencies in IFN-induced effector genes and susceptibility to infection

Host locus	Deficiency	Susceptibility to intracellular pathogens	Refs
Human			
CYBA (encoding p22phox)* CYBB (encoding gp91phox)	Autosomal mutation; complete or partial X-linked mutation; complete or partial	*B. cepacia*, *G. bethesdensis*, *M. tuberculosis*, *S. aureus* *S. marcescens*, *Salmonella* spp.	28,33
IRGM	Autosomal mutation; polymorphic	AIEC, *M. tuberculosis*	66–69
MX1	Autosomal mutation; polymorphic	HBVB7, HCV, measles virus	128
NOS2	Autosomal mutation; polymorphic	*M. tuberculosis*	164
SLC11A1 (encoding NRAMP1)	Autosomal mutation; polymorphic	*M. tuberculosis*	83
Mouse			
Cybb (encoding gp91phox)	X-linked mutation; complete	*A. baumannii*, *A. phagocytophila*, *G. bethesdensis* *H. pylori*, *L. monocytogenes*, *S. aureus*, S. Typhimurium	28,40,41
Gbp1	Autosomal mutation; complete	*L. monocytogenes*, *M. bovis* BCG, S. Typhimurium‡	16
Gbp5	Autosomal mutation; complete	*L. monocytogenes*§	
Ifitm3	Autosomal mutation; complete	Influenza A virus	13
Irgm1	Autosomal mutation; complete	*C. trachomatis*, *L. monocytogenes*, *L. pneumophila* *M. bovis* BCG, *M. tuberculosis*, *S.* Typhimurium, *T. gondii* *T. cruzi*	22,52,61, 95,102
Irgm2	Autosomal mutation; complete	*C. psittaci*	58
Irgm3	Autosomal mutation; complete	*C. trachomatis*, *T. gondii*	56,99
Irga6	Autosomal mutation; complete	*T. gondii*	101
Irgb10	Autosomal mutation; partial	*C. trachomatis*, *C. psittaci*	56,58
Irgd	Autosomal mutation; complete	*T. gondii*	95
Isg15	Autosomal mutation; complete	HSV-1, murine gammaherpesvirus 68, influenza A virus, Sindbisvirus	139
Mx1	Autosomal mutation; complete or polymorphic	Influenza A virus, influenza B virus, Thogoto virus	128
Nos2	Autosomal mutation; complete	*C. trachomatis*, coxsackie B3 virus, ectromelia virus, *L. major*, *L. monocytogenes*, *M. tuberculosis*, *P. yoelli* *S.* Typhimurium, *T. cruzi*, *T. gondii*	7,39,41,91, 93,94,144
Prkra (encoding PKR)	Autosomal mutation; complete	Vaccina virus, West Nilevirus	8
Rnasel (encoding RNase L)or OA Sloci	Autosomal mutation; complete	*B. anthracis*, *E. coli*, HSV-1, vaccinia virus, West Nile virus	8,165
Rsad2 (encoding viperin)	Autosomal mutation; complete	West Nile virus	153

Host locus	Deficiency	Susceptibility to intracellular pathogens	Refs
Slc11a1 (encoding NRAMP1)	Autosomal mutation; complete or polymorphic (*Nramp1*^{G169D})	*C. jejuni*, *L. donovani*, *L. major*, *M.* avium, *M. bovis* BCG, S. Typhimurium	83

AIEC, adherent invasive Escherichia coli; GBP, guanylate-binding protein; HBV57, hepatitis B virus 57; HCV, hepatitis C virus; IFITM, IFN-inducible transmembrane protein; IRG, immunity-related GTPase; ISG15, IFN-stimulated gene 15 kDa protein; MX1, myxovirus resistance 1; NOS2, nitric oxide synthase 2; N RAMP1, natural resistance-associated macrophage protein 1; OAS, 2'–5'oligoadenylate synthase; PKR, IFN-induced, RNA-activated protein kinase.

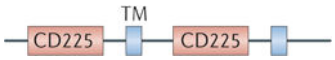
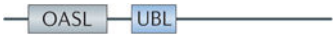
* Other NADPH oxidase components are also affected (p47phox, p67phox and p40phox).

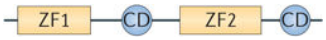
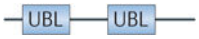
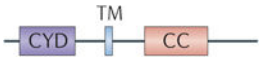
‡ C. J. Bradfield and J.D.M., unpublished observations.

§ A. R. Shenoy and J.D.M., unpublished observations.

Table 3

Repertoire of IFN-induced antiviral effectors

IFN-induced effector family	Name	Domain structure
IFITM family	IFITM1, IFITM2 or IFITM3	
	IFITM5	
TRIM family	Subfamily TRIM C-I	
	Subfamily TRIM C-III	
	Subfamily TRIM C-IV	
	Subfamily TRIM C-V	
	Subfamily TRIM C-VI	
	Subfamily TRIM C-VII	
	Subfamily TRIM C-IX	
	Subfamily TRIM C-X	
	Subfamily TRIM C-II	
	Subfamily TRIM C-VIII	
	Subfamily TRIM C-XI	
MX family	MX1 or MX2	
OAS family	OAS1	
	OAS2	
	OAS3	
	OASL	
PKR	EIF2AK	

IFN-induced effector family	Name	Domain structure
RNase L	RNAseL	
APOBEC3	APOBEC3	
SAMHD1	SAMHD1	
ISG15	ISG15	
Tetherin	Tetherin	
Viperin	Viperin	

Family and domain organization of the major IFN-induced antiviral effectors (see REF. 8). ANK, ankyrin repeats; APOBEC3, apolipoprotein B mRNA-editing enzyme, catalytic polypeptide 3; ARF, ADP ribosylation factor; BB, B-box; BR, bromodomain; CBD, Ca^{2+}-binding domain; CC, coiled-coil domain; CD, cytidine deaminase domain; CID, central interactive domain; COS, C-terminal subgroup one signature; CYD, cytoplasmic domain; DYN, dynamin-like domain; EIF2AK, eukaryotic translation initiation factor 2α kinase; FN3, fibronectin type 3; FIL, filamin-type immunoglobulin; HD, helical domain; IFITM, interferon-inducible transmembrane protein; ISG15, IFN-stimulated gene 15 kDa protein; LZ, leucine zipper; MATH, meprin and TNFR-associated factor homology; MX, myxoma resistance protein; NHL, NHL repeat; OAS, 2'-5' oligoadenylate synthetase domain (catalytically inactive domains shown in grey); P, palmitoylation site; PHD, plant homeodomain; PKR, IFN-induced, RNA-activated protein kinase; PUG, protein kinase domain (containing a UBA or UBx domain); R, RING domain; RBM, RNA binding motif; SAM, radical S-adenosyl methionine domain; SAMHD1, SAM-domain- and HD-domain-containing protein 1; STYK, Ser/Thr/Tyr kinase domain; TM, transmembrane domain; TRIM, tripartite motif protein; UBL, ubiquitin-like domain, ZF, zinc finger.